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NEW APPLICATION TRANSMITTAL FORM



To the Assistant Commissioner for Patents:

This is a Request for filing a non-provisional patent application under 37 CFR 1.53(b) entitled METHODS AND COMPOSITIONS FOR THE TREATMENT OF PANCREATITIS by the following named inventors:

|   |                           |   |   |                                |                    |
|---|---------------------------|---|---|--------------------------------|--------------------|
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(X) The Commissioner is hereby authorized to use Deposit Account Number 01-0885 for the payment of any extension fees incurred during the prosecution of this application.

(X) Enclosed is a specification of 31 pages, claims 7 pages, abstract 1 page, sequence listing 7 pages.

Oath or Declaration

(X) Enclosed is an executed oath or declaration.

() Enclosed is an unsigned oath or declaration.

(X) A self-addressed return postcard is enclosed for verification of receipt.

(X) The filing fee is calculated below:

| FOR  | NUMBER<br>FILED | NUMBER<br>EXTRA | RATE   | FEE            |
|--|-----------------|-----------------|--------|----------------|
| Basic Fee (Large entity)   |                 |                 | \$760  | \$760.00       |
| Total Claims   | 40 minus 20     | = 20            | × \$18 | 360.00         |
| Independent Claims   | 2 minus 3       | = 0             | × \$78 | .00            |
| If application contains any multiple dependent claims, then add \$260.00 |                 |                 |        |                |
| <b>TOTAL FILING FEE</b>  |                 |                 |        | <b>1120.00</b> |

- (X) The Commissioner is hereby authorized to charge the filing fee and excess claim fees (including multiple dependent claim fee) as stated above to Deposit Account No. 01-0885. If this amount is incorrect, or for payment of any other fees that may be incurred as a result of this communication please use said Deposit Account. A duplicate copy of this sheet is enclosed for that purpose.
- () A copy of an assignment bestowing all interest in this application to Allergan Sales, Inc is enclosed.
- () New drawings are enclosed in \_\_ sheets.
- (X) A Statement Pursuant to 37 CFR 1.821(f) and a labeled diskette containing the computer readable sequence listing is enclosed.
- () A Statement Pursuant to 37 CFR § 1.821(e), stating that the paper copy and the computer readable form are identical is filed herewith.
- (X) A properly labeled computer readable form of the Sequence Listing accompanies this Application.
- (X) The Power of Attorney in this application is to Carlos A. Fisher, Registration Number 36,510.
- (X) The Power of Attorney appears in the combined Declaration and Power of Attorney, filed herewith.
- () A copy of the Request for Extension of Time filed in the prior application is enclosed.

Aoki & Sachs

Docket No. 17282(AOC)

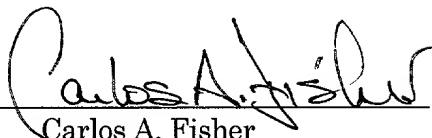
PATENT

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Date: 4/8/99

  
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Aoki & Sachs  
Docket No. 17282

CERTIFICATION UNDER 37 CFR 1.10

I hereby certify that this paper and any documents referred to as enclosed or attached are being deposited with the United States Postal Service on this date in an envelope as "Express Mail Post Office to Addressee" Mailing Label Number EL001807147US addressed to:

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Carlos A. Fisher  
Signature of Person Making Deposit

Carlos A. Fisher  
Printed Name of Person Making Deposit

Applicant: Sachs et al

Title: METHODS AND COMPOSITIONS FOR THE TREATMENT OF PANCREATITIS  
Allergan Docket: 17282(AOC)

Enclosed Are:

Certification Under 37 CFR 1.10 (Express Mail Label No.  
EL001807147US

1. POSTCARD
2. NEW APPLICATION TRANSMITTAL LETTER IN DUPLICATE
3. SPECIFICATION (31 PAGES), CLAIMS (7 PAGES), ABSTRACT (1 PAGE)
4. DECLARATION, POWER OF ATTORNEY
5. STATEMENT 37 CFR § 1.821(f)
6. SEQUENCE LISTING (7 PAGES)
7. DISKETTE

5 METHODS AND COMPOSITIONS  
FOR THE TREATMENT OF PANCREATITIS

10 Field of the Invention

The present invention includes methods and compositions for the treatment of acute pancreatitis. In a preferred embodiment the invention concerns the use of agents to  
15 reduce or prevent the secretion of pancreatic digestive enzymes within the pancreas. Such agents are targeted to pancreatic cells, and serve to prevent the exocytotic fusion of vesicles containing these enzymes with the plasma membrane. The invention is also concerned with methods of  
20 treating a mammal suffering from pancreatitis through the administration of such agents.

Background of the Invention

25 Pancreatitis is a serious medical condition involving an inflammation of the pancreas. In acute or chronic pancreatitis the inflammation manifests itself in the release and activation of pancreatic enzymes within the organ itself, leading to autodigestion. In many cases of  
30 acute pancreatitis, the condition can lead to death.

In normal mammals, the pancreas, a large gland similar in structure to the salivary gland, is responsible for the production and secretion of digestive enzymes, which digest ingested food, and bicarbonate for the neutralization of the  
35 acidic chyme produced in the stomach. The pancreas contains

5 acinar cells, responsible for enzyme production, and ductal cells, which secrete large amounts of sodium bicarbonate solution. The combined secretion product is termed "pancreatic juice"; this liquid flows through the pancreatic duct past the sphincter of Oddi into the duodenum. The  
10 secretion of pancreatic juice is stimulated by the presence of chyme in the upper portions of the small intestine, and the precise composition of pancreatic juice appears to be influenced by the types of compounds (carbohydrate, lipid, protein, and/or nucleic acid) in the chyme.

15 The constituents of pancreatic juice includes proteases (trypsin, chymotrypsin, carboxypolypeptidase), nucleases (RNase and DNase), pancreatic amylase, and lipases (pancreatic lipase, cholesterol esterase and phospholipase).

Many of these enzymes, including the proteases, are  
20 initially synthesized by the acinar cells in an inactive form as zymogens: thus trypsin is synthesized as trypsinogen, chymotrypsin as chymotrypsinogen, and carboxypolypeptidase as procarboxypolypeptidase. These enzymes are activated according to a cascade, wherein, in  
25 the first step, trypsin is activated through proteolytic cleavage by the enzyme enterokinase. Trypsinogen can also be autoactivated by trypsin; thus one activation has begun, the activation process can proceed rapidly. Trypsin, in turn, activates both chymotrypsinogen and  
30 procarboxypolypeptidase to form their active protease counterparts.

The enzymes are normally activated only when they enter the intestinal mucosa in order to prevent autodigestion of the pancreas. In order to prevent premature activation, the

5 acinar cells also co-secrete a trypsin inhibitor that normally prevents activation of the proteolytic enzymes within the secretory cells and in the ducts of the pancreas. Inhibition of trypsin activity also prevents activation of the other proteases.

10 Pancreatitis can occur when an excess amount of trypsin saturates the supply of trypsin inhibitor. This, in turn, can be caused by underproduction of trypsin inhibitor, or the overabundance of trypsin within the cells or ducts of the pancreas. In the latter case, pancreatic trauma or  
15 blockage of a duct can lead to localized overabundance of trypsin; under acute conditions large amounts of pancreatic zymogen secretion can pool in the damaged areas of the pancreas. If even a small amount of free trypsin is available activation of all the zymogenic proteases rapidly  
20 occurs, and can lead to digestion of the pancreas (acute pancreatitis) and in particularly severe cases to the patient's death.

Pancreatic secretion is normally regulated by both hormonal and nervous mechanisms. When the gastric phase of  
25 stomach secretion occurs, parasympathetic nerve impulses are relayed to the pancreas, which initially results in acetylcholine release, followed by secretion of enzymes into the pancreatic acini for temporary storage.

When acid chyme thereafter enters the small intestine,  
30 the mucosal cells of the upper intestine release a hormone called secretin. In humans, secretin is a 27 amino acid (3400 Dalton) polypeptide initially produced as the inactive form prosecretin, which is then activated by proteolytic cleavage. Secretin is then absorbed into the blood.

5 Secretin causes the pancreas to secrete large quantities of  
a fluid containing bicarbonate ion. Secretin does not  
stimulate the acinar cells, which produce the digestive  
enzymes. The bicarbonate fluid serves to neutralize the  
chyme and to provide a slightly alkaline optimal environment  
10 for the enzymes.

Another peptide hormone, cholecystokinin (CCK) is  
released by the mucosal cells in response to the presence of  
food in the upper intestine. As described in further detail  
below, human CCK is synthesized as a protoprotein of 115  
15 amino acids. Active CCK forms are quickly taken into the  
blood through the digestive tract, and normally stimulate  
the secretion of enzymes by the acinar cells. However,  
stimulation of the CCK receptor by the CCK analogs cerulein  
and CCK-octapeptide (CCK-8) appears to lead to a worsening  
20 of morbidity and mortality in mammals in whom pancreatitis  
is induced. See Tani et al., *Pancreas* 5:284-290 (1990).

As indicated above, the digestive enzymes are  
synthesized as zymogens; proto-enzyme synthesis occurs in  
the rough endoplasmic reticulum of the acinar cells. The  
25 zymogens are then packaged within vesicles having a single  
lipid bilayer membrane. The zymogens are packed within the  
vesicles so densely that they appear as quasi-crystalline  
structures when observed under light microscopy and the  
zymogen granules are electron-dense when observed under the  
30 electron microscope. The vesicles are localized within the  
cytoplasm of the acinar cells. Secretion of zymogens by the  
acinar cells occurs through vesicle docking and subsequent  
fusion with the plasma membrane, resulting in the liberation  
of the contents into the extracellular milieu.



5 Nerve cells appear to secrete neurotransmitters and other intercellular signaling factors through a mechanism of membrane fusion that is shared with other cell types, see e.g., Rizo & Sudhof, *Nature Struct. Biol.* 5:839-842 (October 1998), hereby incorporated by reference herein,  
10 including the pancreatic acinar cells.

Although the Applicants do not wish to be bound by theory, it is believed that a vesicle first contacts the intracellular surface of the cellular membrane in a reaction called docking. Following the docking step the membrane  
15 fuses with and becomes part of the plasma membrane through a series of steps that currently remain relatively uncharacterized, but which clearly involve certain vesicle and membrane-associated proteins, as has been illustrated using neural models.

20 In neurons, neurotransmitters are packaged within synaptic vesicles, formed within the cytoplasm, then transported to the inner plasma membrane where the vesicles dock and fuse with the plasma membrane. Recent studies of nerve cells employing clostridial neurotoxins as probes of  
25 membrane fusion have revealed that fusion of synaptic vesicles with the cell membrane in nerve cells depends upon the presence of specific proteins that are associated with either the vesicle or the target membrane. *See id.* These proteins have been termed SNAREs. As discussed in further  
30 detail below, a protein alternatively termed synaptobrevin or VAMP (vesicle-associated membrane protein) is a vesicle-associated SNARE (v-SNARE). There are at least two isoforms of synaptobrevin; these two isoforms are differentially expressed in the mammalian central nervous system, and are

5 selectively associated with synaptic vesicles in neurons and  
secretory organelles in neuroendocrine cells. The target  
membrane-associated SNAREs (t-SNAREs) include syntaxin and  
SNAP-25. Following docking, the VAMP protein forms a core  
10 complex appears to be an essential step to membrane fusion.  
See Rizo & Sudhof, *id.* and Neimmann et al., *Trends in Cell  
Biol.* 4:179-185 (May 1994), hereby incorporated by  
referenced herein.

15 Recently evidence has increasingly indicated that  
the SNARE system first identified in neural cells is a  
general model for membrane fusion in eukaryotic cells. A  
yeast exocytotic core complex similar to that of the  
synaptic vesicles of mammalian neural cells has been  
characterized, and found to contain three proteins: Sso 1  
20 (syntaxin 1 homolog), SncI (synaptobrevin homolog), and sec9  
(SNAP-25 homolog). Rizo & Sudhof, *id.* These proteins share  
a high degree of amino acid sequence homology with their  
mammalian synaptosomal counterparts.

25 All mammalian non-neuronal cells appear to contain  
cellubrevin, a synaptobrevin analog - this protein is  
involved in the intracellular transport of vesicles, and is  
cleaved by TeTx, BoNT/E, BoNT/F, and BoNT/G. Homologs of  
syntaxin have been identified in yeast (e.g., sso1p and  
sso2p) and mammalian non-neuronal cells (syn2p, syn3p, syn4p  
30 and syn5p). Finally, as indicated above, a yeast SNAP-25  
homolog, sec9 has been identified; this protein appears to  
essential for vesicle fusion with the plasma membrane.

Intoxication of neural cells by clostridial  
neurotoxins exploits specific characteristics of the SNARE

5 proteins. These neurotoxins, most commonly found expressed  
in *Clostridium botulinum* and *Clostridium tetanus*, are highly  
potent and specific poisons of neural cells. These Gram  
positive bacteria secrete two related but distinct toxins,  
each comprising two disulfide-linked amino acid chains: a  
10 light chain (L) of about 50 KDa and a heavy chain (H) of  
about 100 KDa, which are wholly responsible for the symptoms  
of botulism and tetanus, respectively.

The tetanus and botulinum toxins are among the most  
lethal substances known to man; both toxins function by  
15 inhibiting neurotransmitter release in affected neurons.  
The tetanus neurotoxin (TeNT) acts mainly in the central  
nervous system, while botulinum neurotoxin (BoNT) acts at  
the neuromuscular junction; both toxins inhibit  
acetylcholine release from the nerve terminal of the  
20 affected neuron into the synapse, resulting in paralysis or  
reduced target organ function.

The tetanus neurotoxin (TeNT) is known to exist in one  
immunologically distinct type; the botulinum neurotoxins  
(BoNT) are known to occur in seven different immunologically  
25 distinct serotypes, termed BoNT/A through BoNT/G. While all  
of these latter types are produced by isolates of *C.*  
*botulinum*, two other species, *C. baratii* and *C. butyricum*  
also produce toxins similar to /F and /E, respectively. See  
e.g., Coffield et al., *The Site and Mechanism of Action of*  
30 *Botulinum Neurotoxin in Therapy with Botulinum Toxin* 3-13  
(Jankovic J. & Hallett M. eds. 1994), the disclosure of  
which is incorporated herein by reference.

Regardless of type, the molecular mechanism of  
intoxication appears to be similar. In the first step of

5 the process, the toxin binds to the presynaptic membrane of  
the target neuron through a specific interaction between the  
heavy chain and a neuronal cell surface receptor; the  
receptor is thought to be different for each type of  
botulinum toxin and for TeNT. The carboxy terminal (C-  
10 terminal) half of the heavy chain is required for targeting  
of the toxin to the cell surface. The cell surface  
receptors, while not yet conclusively identified, appear to  
be distinct for each neurotoxin serotype.

In the second step, the toxin crosses the plasma  
15 membrane of the poisoned cell. The toxin is first engulfed  
by the cell through receptor-mediated endocytosis, and an  
endosome containing the toxin is formed. The toxin (or  
light chain thereof) then escapes the endosome into the  
cytoplasm of the cell. This last step is thought to be  
20 mediated by the amino terminal (N-terminal) half of the  
heavy chain, which triggers a conformational change of the  
toxin in response to a pH of about 5.5 or lower. Endosomes  
are known to possess a proton pump that decreases intra-  
endosomal pH. The conformational shift exposes hydrophobic  
25 residues in the toxin, which permits the toxin to embed  
itself in the endosomal membrane. The toxin then  
translocates through the endosomal membrane into the  
cytosol.

Either during or after translocation the disulfide bond  
30 joining the heavy and light chain is reduced, and the light  
chain is released into the cytoplasm. The entire toxic  
activity of botulinum and tetanus toxins is contained in the  
light chain of the holotoxin; the light chain is a zinc  
(Zn++) endopeptidase which selectively cleaves the SNARE

5 proteins essential for recognition and docking of  
neurotransmitter-containing vesicles with the cytoplasmic  
surface of the plasma membrane, and fusion of the vesicles  
with the plasma membrane. The light chain of TxNT, BoNT/B,  
BoNT/D, BoNT/F, and BoNT/G cause specific proteolysis of  
10 VAMP, an integral protein. During proteolysis, most of the  
VAMP present at the cytosolic surface of the synaptic  
vesicle is inactivated as a result of any one of these  
cleavage events. Each toxin cleaves a different specific  
peptide bond.

15 BoNT/A and /E selectively cleave the plasma membrane-  
associated SNARE protein SNAP-25; this protein is bound to  
and present on the cytoplasmic surface of the plasma  
membrane. BoNT/C1 cleaves syntaxin, which exists as an  
integral protein having most of its mass exposed to the  
20 cytosol. Syntaxin interacts with the calcium channels at  
presynaptic terminal active zones. See Tonello et al.,  
*Tetanus and Botulism Neurotoxins in Intracellular Protein  
Catabolism* 251-260 (Suzuki K & Bond J. eds. 1996), the  
disclosure of which is incorporated by reference as part of  
25 this specification. Bo/NTC1 also appears to cleave SNAP-25.

Both TeNT and BoNT are specifically taken up by cells  
present at the neuromuscular junction. BoNT remains within  
peripheral neurons and, as indicated above, blocks release  
of the neurotransmitter acetylcholine from these cells.

30 By contrast TeNT, through its receptor, enters vesicles  
that move in a retrograde manner along the axon to the soma,  
and is discharged into the intersynaptic space between motor  
neurons and the inhibitory neurons of the spinal cord. At  
this point, TeNT binds receptors of the inhibitory neurons,

5 is again internalized, and the light chain enters the  
cytosol to block the release of the inhibitory  
neurotransmitters 4-aminobutyric acid (GABA) and glycine  
from these cells. Id.

International Patent Publication No. WO 96/33273  
10 relates to derivatives of botulinum toxin designed to  
prevent neurotransmitter release from sensory afferent  
neurons to treat chronic pain. Such derivatives are  
targeted to nociceptive neurons using a targeting moiety  
that binds to a binding site of the surface of the neuron.

15 International Patent Publication No. 98/07864 discusses  
the production of recombinant toxin fragments that have  
domains that enable the polypeptide to translocate into a  
target cell or which increase the solubility of the  
polypeptide, or both.

20  
Summary of the Invention

The present invention concerns methods and compositions  
25 useful for the treatment of acute pancreatitis. This  
condition is largely due to the defective secretion of  
zymogen granules by acinar cells, and by the premature co-  
mingling of the secreted zymogens with lysosomal  
hydrolysates capable of activating trypsin, thereby  
30 triggering the protease activation cascade and resulting in  
the destruction of pancreatic tissue.

In one embodiment of this aspect, the invention is a  
therapeutic agent comprising a chimeric protein containing  
an amino acid sequence-specific endopeptidase activity which

5 will specifically cleave at least one synaptic vesicle-  
associated protein selected from the group consisting of  
SNAP-25, syntaxin or VAMP, in combination with the  
translocation activity of the N-terminus of a clostridial  
neurotoxin heavy chain, wherein the chimeric protein further  
10 comprises a recognition domain which will bind a human  
cholecystokinin (CCK) receptor. Upon binding of the  
recognition domain of the protein to the CCK receptor, the  
protein is specifically transported into cells containing  
CCK receptors (pancreatic acinar cells) through receptor-  
15 mediated endocytosis. In a preferred embodiment, the CCK  
receptor is the CCK A receptor.

Once inside the acinar cell, the chimeric protein  
functions in a manner similar to that of a clostridial  
neurotoxin within its target neuron. The toxin moiety is  
20 translocated from the endosome into the cytoplasm, where it  
acts to cleave a SNARE protein identical or homologous to  
SNAP-25, syntaxin or VAMP. The cleavage of this protein  
prevents formation of a core complex between the SNARE  
proteins and thus prevents or reduces the extent of fusion  
25 of the vesicle with the target membrane. This, in turn,  
results in inhibition of zymogen release from the acinar  
cells and of zymogen activation by lysosomal hydrolases.  
The autodigestion of pancreatic tissue in acute pancreatitis  
is therefore reduced or eliminated.

30 Another embodiment of the present invention concerns a  
method of treating a patient suffering from acute  
pancreatitis by administering an effective amount of such a  
chimeric protein.

Another embodiment of the invention concerns a

5 therapeutic composition that contains the translocation  
activity of a clostridial neurotoxin heavy chain in  
combination with a recognition domain able to bind a  
specific cell type and a therapeutic element having an  
activity other than the endopeptidase activity of a  
10 clostridial neurotoxin light chain. A non-exclusive list of  
certain such therapeutic elements includes: hormones and  
hormone-agonists and antagonists, nucleic acids capable  
being of being used as replication, transcription, or  
translational templates (e.g., for expression of a protein  
15 drug having the desired biological activity or for synthesis  
of a nucleic acid drug as an antisense agent), enzymes,  
toxins, and the like.

In a preferred embodiment, the specific cell type is a  
pancreatic cell, most preferably a pancreatic acinar cell.

20 Another embodiment is drawn to methods for the  
treatment of acute pancreatitis comprising contacting an  
acinar cell with an effective amount of a composition  
comprising a chimeric protein containing an amino acid  
sequence-specific endopeptidase activity which will  
25 specifically cleave at least one synaptic vesicle-associated  
protein selected from the group consisting of SNAP-25,  
syntaxin or VAMP, in combination with the translocation  
activity of the N-terminus of a clostridial neurotoxin heavy  
chain, wherein the chimeric protein further comprises a  
30 recognition domain able to bind to a cell surface protein  
characteristic of an human pancreatic acinar cell.  
Preferably the cell surface protein is a CCK receptor  
protein; most preferably the protein is the human CCK A  
protein. CCK receptors (CCK-A receptor and CCK-B receptor)



5 are found mainly in on the surface of pancreatic acinar cells, although they are also found in some brain cells and, to a lesser extent on the surface of gastrointestinal cells.

Any suitable route of administration may be used in this aspect of the invention. Applicants currently prefer  
10 to administer the therapeutic agent in an intravenous infusion solution; however methods such as ingestion (particularly when associated with neurotoxin-associated proteins (NAPs); see Sharma et al., *J. Nat. Toxins* 7:239-253(1998), incorporated by reference herein), direct  
15 delivery to the pancreas, injection and the like may also be used. The agent is substantially specifically targeted to pancreatic cells; when the agent contains a CCK receptor-binding domain, the blood-brain barrier prevents the agent from interacting with brain cells.

20 In yet another embodiment the invention provides a composition comprising a drug or other therapeutic agent having an activity other than that of a clostridial neurotoxin light chain for intracellular delivery, said agent joined to the translocation domain of a clostridial  
25 neurotoxin heavy chain and a binding element able to recognize a cell surface receptor of a target cell. In a preferred embodiment, the target cell is not a neuron. Also, in this embodiment it is preferred that the drug or other therapeutic agent has an enzymatic, catalytic, or  
30 other self-perpetuating mode of activity, so that the effective dose of drug is greater than the number of drug molecules delivered within the target cell. A non-exclusive list of certain such drugs would include: hormones and hormone-agonists and antagonists, nucleic acids capable

5 being of being used as replication, transcription, or translational templates (e.g., for expression of a protein drug having the desired biological activity or for synthesis of a nucleic acid drug as an antisense agent), enzymes, toxins (such as diphtheria toxin or ricin), and the like.

10 In this embodiment the drug may be cleavably linked to the remainder of the composition in such a way as to allow for the release of the drug from the composition within the target cell.

The presently claimed compositions may be provided to  
15 the patient by intravenous administration, may be administered during surgery, or may be provided parenterally.

WO 95/32738, which is shares ownership with the present application, describes transport proteins for the  
20 therapeutic treatment of neural cells. This application is incorporated by reference herein as part of this specification.

#### Detailed Description of the Preferred Embodiments

25 In a basic and presently preferred form, the invention comprises a therapeutic polypeptide comprising three features: a binding element, a translocation element, and a therapeutic element.

30 The binding element is able to bind to a specific target cell provided that the target cell is not a motor neuron or a sensory afferent neuron. Preferably, the binding element comprises an amino acid chain; also an independently, it is preferably located at or near the C-

5 terminus of a polypeptide chain. By "binding element" is  
meant a chemical moiety able to preferentially bind to a  
cell surface marker characteristic of the target cell under  
physiological conditions. The cell surface marker may  
comprise a polypeptide, a polysaccharide, a lipid, a  
10 glycoprotein, a lipoprotein, or may have structural  
characteristics of more than one of these. By  
"preferentially interact" is meant that the disassociation  
constant ( $K_d$ ) of the binding element for the cell surface  
marker is at least one order of magnitude less than that of  
15 the binding element for any other cell surface marker.  
Preferably, the disassociation constant is at least 2 orders  
of magnitude less, even more preferably the disassociation  
constant is at least 3 orders of magnitude less than that of  
the binding element for any other cell surface marker to  
20 which the therapeutic polypeptide is exposed. Preferably,  
the organism to be treated is a human.

In one embodiment the cell surface receptor comprises  
the histamine receptor, and the binding element comprises an  
variable region of an antibody which will specifically bind  
25 the histamine receptor.

In an especially preferred embodiment, the cell surface  
marker is a cholecystokinin (CCK) receptor. Cholecystokinin  
is a bioactive peptide that functions as both a hormone and  
a neurotransmitter in a wide variety of physiological  
30 settings. Thus, CCK is involved in the regulation of gall  
bladder contraction, satiety, gastric emptying, and gut  
motility; additionally it is involved in the regulation of  
pancreatic exocrine secretion.

5           There are two types of CCK receptors, CCK A and CCK B;  
the amino acid sequences of these receptors have been  
determined from cloned cDNA. Despite the fact that both  
receptors are G protein-coupled receptors and share  
approximately 50% homology, there are distinct differences  
10 between their physiological activity. The CCK A receptor is  
expressed in smooth muscle cells of the gall bladder, smooth  
muscle and neurons within the gastrointestinal tract, and  
has a much greater affinity ( $>10^2$  times higher) for CCK than  
the related peptide hormone gastrin. The CCK B receptor,  
15 found in the stomach and throughout the CNS, has roughly  
equal ability to bind CCK and gastrin.

The varied activities of CCK can be partly attributed  
to the fact that CCK is synthesized as procholecystokinin, a  
protoprotein of 115 amino acids, and is then post-  
20 translationally cleaved into a number of active fragments  
all sharing the same C-terminus. The amino acid sequence of  
human procholecystokinin is shown below; amino acid residues  
not present in the biologically active cleavage products are  
in lower case. All amino acid sequences herein are shown  
25 from N-terminus to C-terminus, unless expressly indicated  
otherwise:

Human procholecystokinin, having the amino acid  
sequence SEQ ID NO:1:

30           mmsgvclclvmlavlaagaltqpvp padpagsglqraeeaprrqlr VSQRT  
DGESRAHLGA LLARYIQQAR KAPSGRMSIV KNLQNLDP SH RISDRDYMGW  
MDF grrsaeeyeyps

5           Biologically active cleavage products of the full  
length CCK chain include:

CCK-58, having the amino acid sequence SEQ ID NO:2:

VSQRT DGESRAHLGA LLARYIQQAR KAPSGRMSIV KNLQNLDP SH  
10       RISDRDYM GW MDF;

CCK-39, having the amino acid sequence SEQ ID NO: 3:

YIQQAR KAPSGRMSIV KNLQNLDP SH RISDRDYM GW MDF;  
15

CCK-33, having the amino acid sequence SEQ ID NO: 4:

KAPSGRMSIV KNLQNLDP SH RISDRDYM GW MDF;

20       CCK-12, having the amino acid sequence SEQ ID NO: 5:

ISDRDYM GW MDF;

and CCK-8, having the amino acid sequence SEQ ID NO: 6:

25       RDYM GW MDF.

In each case, the biologically active polypeptides  
contain two additional post-translational modifications;  
amidation of the C-terminal phenylalanine, and sulfatation  
30 of the aspartic acid residue located seven residue from the  
C-terminus of the biologically active species. These  
modifications appear to be necessary for full biological  
activity, although both the C-terminal pentapeptide and  
tetrapeptide of CCK retains some biological activity.

5 Kennedy et al., *J. Biol. Chem.* 272: 2920-2926 (1997), hereby incorporated by reference herein.

While it will be understood that the applicants do not wish to be bound by theory, the following findings may assist an understanding the nature of the interaction  
10 between CCK and the CCK receptors, and thus between the CCK receptor binding element of an embodiment of the present invention and its CCK receptor target.

In pancreatic acinar cells the CCK A receptor undergoes internalization to intracellular sites within minutes after  
15 agonist exposure. Pohl et al., *J. Biol. Chem.* 272: 18179-18184 (1997), hereby incorporated by reference herein. The CCK B receptor has also shown the same ligand-dependant internalization response in transfected NIH 3T3 cells. In the CCK B receptor, but not the CCK A receptor, the  
20 endocytotic feature of the receptor been shown to be profoundly decreased by the deletion of the C terminal 44 amino acids of the receptor chain, corresponding in both receptors to an cytoplasmic portion of the receptor chain.

Recent studies of the interaction between the CCK A  
25 receptor and CCK have shown that the primary receptor sequence region containing amino acid residues 38 through 42 is involved in the binding of CCK. Residues Trp<sub>39</sub> and Gln<sub>40</sub> appear to be essential for the binding of a synthetic CCK C-terminal nonapeptide (in which the methionine residues  
30 located at residue 3 and 6 from the C-terminus are substituted by norleucine and threonine respectively) to the receptor. Kennedy et al., *supra*. These residues do not appear to be essential for the binding of CCK analogs JMV 180 (corresponding the synthetic C-terminal heptapeptide of

5 CCK in which the phenylalanylamide residue is substituted by  
a phenylethyl ester and the threonine is substituted with  
norleucine), and JMV 179 (in which the phenylalanylamide  
residue and the L-tryptophan residues of the synthetic CCK  
nonapeptide are substituted by a phenylethyl ester and D-  
10 tryptophan, respectively and the threonine is substituted  
with norleucine). *Id.*

These and similar studies have shed light on the  
structure of the CCK A receptor active site. Based on  
receptor binding experiments, a current structural model  
15 indicates that CCK residues Trp<sub>30</sub> and Met<sub>31</sub> (located at  
positions 4 and 3, respectively, from the C terminus of  
mature CCK-8) reside in a hydrophobic pocket formed by  
receptor residues Leu<sub>348</sub>, Pro<sub>352</sub>, Ile<sub>353</sub> and Ile<sub>356</sub>. CCK residue  
Asp<sub>32</sub> (located at amino acid position 2 measured from the C  
20 terminus of CCK-8) seems to be involved in an ionic  
interaction with receptor residue Lys<sub>115</sub>. CCK Tyr-sulfate<sub>27</sub>  
(the CCK-8 residue 7 amino acids from C terminus) appears  
involved in an ionic interaction with receptor residue Lys<sub>105</sub>  
and a stacking interaction with receptor residue Phe<sub>198</sub>. Ji,  
25 et al., 272 *J. Biol. Chem.* 24393-24401 (1997).

Such structural models provide detailed guidance to the  
person of ordinary skill in the art as to the construction  
of a variety of binding elements able to retain the binding  
characteristics of biologically active CCK peptides for the  
30 CCK-A receptor, for example, as, for example, by site  
directed mutagenesis of a clostridial neurotoxin heavy  
chain. Similarly, models deduced using similar methodologies  
have been proposed for the CCK B receptor, see e.g.,

5 Jagerschmidt, A. et al., *Mol. Pharmacol.* 48:783-789 (1995),  
and can be used as a basis for the construction of binding  
elements that retain binding characteristics similar to the  
CCK B receptor.

10 Additionally, the binding element may comprise a  
variable region of an antibody which will bind the CCK-A or  
CCK-B receptor.

15 Nucleic acids encoding polypeptides containing such a  
binding element may be constructed using molecular biology  
methods well known in the art; see e.g., Sambrook et al.,  
*Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor  
Laboratory Press 2d ed. 1989), and expressed within a  
suitable host cell. The disclosure of this latter reference  
is incorporated by reference herein in its entirety.

20 The translocation element comprises a portion of a  
clostridial neurotoxin heavy chain having a translocation  
activity. By "translocation" is meant the ability to  
facilitate the transport of a polypeptide through a  
vesicular membrane, thereby exposing some or all of the  
polypeptide to the cytoplasm.

25 In the various botulinum neurotoxins translocation is  
thought to involve an allosteric conformational change of  
the heavy chain caused by a decrease in pH within the  
endosome.

30 This conformational change appears to involve and be  
mediated by the N terminal half of the heavy chain and to  
result in the formation of pores in the vesicular membrane;  
this change permits the movement of the proteolytic light  
chain from within the endosomal vesicle into the cytoplasm.



5 See e.g., Lacy, et al., *Nature Struct. Biol.* 5:898-902  
(October 1998).

The amino acid sequence of the translocation-mediating  
portion of the botulinum neurotoxin heavy chain is known to  
those of skill in the art; additionally, those amino acid  
10 residues within this portion that are known to be essential  
for conferring the translocation activity are also known.

It would therefore be well within the ability of one of  
ordinary skill in the art, for example, to employ the  
naturally occurring N-terminal peptide half of the heavy  
15 chain of any of the various *Clostridium tetanus* or  
*Clostridium botulinum* neurotoxin subtypes as a translocation  
element, or to design an analogous translocation element by  
aligning the primary sequences of the N-terminal halves of  
the various heavy chains and selecting a consensus primary  
20 translocation sequence based on conserved amino acid,  
polarity, steric and hydrophobicity characteristics between  
the sequences.

The therapeutic element of the present invention may  
comprise, without limitation: active or inactive (i.e.,  
25 modified) hormone receptors (such as androgen, estrogen,  
retinoid, peroxisome proliferator and ecdysone receptors  
etc.), and hormone-agonists and antagonists, nucleic acids  
capable being of being used as replication, transcription,  
or translational templates (e.g., for expression of a  
30 protein drug having the desired biological activity or for  
synthesis of a nucleic acid drug as an antisense agent),  
enzymes, toxins (including apoptosis-inducing agents), and  
the like.

5 In a preferred embodiment, the therapeutic element is a polypeptide comprising a clostridial neurotoxin light chain or a portion thereof retaining the SNARE-protein sequence-specific endopeptidase activity of a clostridial neurotoxin light chain. The amino acid sequences of the light chain of  
10 botulinum neurotoxin (BoNT) subtypes A-G have been determined, as has the amino acid sequence of the light chain of the tetanus neurotoxin (TeNT). Each chain contains the Zn<sup>++</sup>-binding motif **His-Glu-x-x-His** (N terminal direction at the left) characteristic of Zn<sup>++</sup>-dependent endopeptidases  
15 (HELIH in TeNT, BoNT/A /B and /E; HELNH in BoNT/C; and HELTH in BoNT/D).

Recent studies of the BoNT/A light chain have revealed certain features important for the activity and specificity of the toxin towards its target substrate, SNAP-25. Thus,  
20 studies by Zhou et al. *Biochemistry* 34:15175-15181 (1995) have indicated that when the light chain amino acid residue His<sub>227</sub> is substituted with tyrosine, the resulting polypeptide is unable to cleave SNAP-25; Hurazono et al., *J. Biol. Chem.* 14721-14729 (1992) performed studies in the  
25 presynaptic cholinergic neurons of the buccal ganglia of *Aplysia californica* using recombinant BoNT/A light chain that indicated that the removal of 10 N-terminal or 32 C-terminal residues did not abolish toxicity, but that removal of 10 N-terminal or 57 C-terminal residues abolished  
30 toxicity in this system. Most recently, the crystal structure of the entire BoNT/A holotoxin has been solved; the active site is indicated as involving the participation of His<sub>222</sub>, Glu<sub>223</sub>, His<sub>226</sub>, Glu<sub>261</sub> and Tyr<sub>365</sub>. Lacy et al., *supra*.

5 (These residues correspond to His<sup>223</sup>, Glu<sup>224</sup>, His<sup>227</sup>, Glu<sup>262</sup> and Tyr<sup>366</sup> of the BoNT/A L chain of Kurazono et al., *supra*.)  
Interestingly, an alignment of BoNT/A through E and TeNT light chains reveals that every such chain invariably has these residues in positions analogous to BoNT/A. Kurazono  
10 et al., *supra*.

The catalytic domain of BoNT/A is very specific for the C-terminus of SNAP-25 and appears to require a minimum of 16 SNAP-25 amino acids for cleavage to occur. The catalytic site resembles a pocket; when the light chain is linked to  
15 the heavy chain via the disulfide bond between Cys<sup>429</sup> and Cys<sup>453</sup>, the translocation domain of the heavy chain appears to block access to the catalytic pocket until the light chain gains entry to the cytosol. When the disulfide bond is reduced, the two polypeptide chains dissociate, and the  
20 catalytic pocket is then "opened" and the light chain is fully active.

As described above, VAMP and syntaxin are cleaved by BoNT/B, D, F, G and TeNT, and BoNT/C<sub>1</sub>, respectively, while SNAP-25 is cleaved by BoNT/A and E.

25 The substrate specificities of the various clostridial neurotoxin light chains other than BoNT/A are known. Therefore, the person of ordinary skill in the art could easily determine the toxin residues essential in these subtypes for cleavage and substrate recognition (for  
30 example, by site-directed mutagenesis or deletion of various regions of the toxin molecule followed by testing of proteolytic activity and substrate specificity), and could

5 therefore easily design variants of the native neurotoxin  
light chain that retain the same or similar activity.

Additionally, construction of the therapeutic agents  
set forth in this specification would be easily constructed  
by the person of skill in the art. It is well known that the  
10 clostridial neurotoxins have three functional domains  
analogous to the three elements of the present invention.  
For example, the BoNT/A neurotoxin light chain is present in  
amino acid residues 1-448 of the BoNT/A prototoxin (i.e.,  
before nicking of the prototoxin to form the disulfide-  
15 linked dichain holotoxin); this amino acid sequence is  
provided below as SEQ ID NO: 7. Active site residues are  
underlined:

BoNT/A light chain (SEQ ID NO:7)

20

MPFVNKQFNYKDPVNGVDIAYIKIPNAGQMOPVKAFKIHNKI WV  
IPERDTFTNPEEGDLNPPPEAKQVPVSYDYSTYLSTDNEKDNYLKGVTCLFERIYSTD  
LGRMLLTISIVRGIPFWGGSTIDTELKVIDTNCINVIQPDGSYRSEELNLVIIGPSADI  
IQFECKSFGHEVLNLTRNGYGSTQYIRFSPDFTFGFEESLEVDTNPLL GAGKFATDPA  
25 VTLAHEL I H A G H R L Y G I A I N P N R V F K V N T N A Y Y E M S G L E V S F E E L R T F G G H D A K F I D S  
LQNEFRRLYYYNKFKDIASTLNKAKSIVGTTASLQYMKNVFKEKYLLSEDTSGKFSVD  
KLKFDKLYKMLTEIYTEDNFVKFFKVLNRKTYLNFDAVFKINIVPKVNYTIYDGFNL  
RNTNLAANFNGQNT E I N N M N F T K L K N F T G L F E F Y K L L C V R G I I T S K T K S L D K G Y N K ;

30 The heavy chain N-terminal (H<sub>N</sub>) translocation domain is  
contained in amino acid residues 449-871 of the BoNT/A amino  
acid sequence, shown below as SEQ ID NO: 8; a gated ion  
channel-forming domain probably essential for the  
translocation activity of this peptide is underlined (see  
35 Oblatt-Montal et al., *Protein Sci.* 4:1490-1497(1995), hereby  
incorporated by reference herein.

5 ALNDLCIKVNNWDLFFSPSEDNFTNDLNKGEEITSDTNIEAAEENISLDLIQQYYLTFNF  
 DNEPENISIENLSSDIIGQLELMPNIERFPNGKKYELDKYTMFHYLRAQEFEGHKSRI  
 ALTNSVNEALLNPSRVYTFSSDYVKKVKNKATEAMFLGWVEQLVYDFTDETSEVSTT  
 DKIAIDITIIIPYIGPALNIGNMLYKDDFVGALIFSGAVILLEFIPEIAIPVLGTFALV  
 SYIANKVLTVQTIDNALS KRNEKWDEVYKYIVTNWLAKVNTQIDLIRKKMKEALENQA  
 10 EATKAIINYQYNQYTEEEKNNINFNIDDLSSKLNESINKAMININKFLNQCSVSYLMN  
 SMIPYGVKRLEDFDASLKDALLKYIYDNRGTLIGQVDRLLKDKVNNTLSTDIPFQLSKY  
 VDNQRLSTFTTEYIK;

The heavy chain C-terminal neural cell binding domain  
 15 is contained in amino acid residues 872-1296 (SEQ ID NO: 9)  
 of the BoNT/A prototoxin.

NIINTSILNLRYESNHLIDLSRYASKINIGSKVNFDPIDKNQI  
 QLFNLESSKIEVILKNAIVYNSMYENFSTSFWIRIPKYFNSISLNNEYTIINCMENNS  
 20 GWKVS LN YG E I I W T L Q D T Q E I K Q R V V F K Y S Q M I N I S D Y I N R W I F V T I T N N R L N N S K I Y  
 I N G R L I D Q K P I S N L G N I H A S N N I M F K L D G C R D T H R Y I W I K Y F N L F D K E L N E K E I K D L Y  
 D N Q S N S G I L K D F W G D Y L Q Y D K P Y M L N L Y D P N K Y V D V N N V G I R G Y M Y L K G P R G S V M T T  
 N I Y L N S S L Y R G T K F I I K K Y A S G N K D N I V R N N D R V Y I N V V V K N K E Y R L A T N A S Q A G V E K  
 I L S A L E I P D V G N L S Q V V M K S K N D Q G I T N K C K M N L Q D N N G N D I G F I G F H Q F N N I A K L V  
 25 A S N W Y N R Q I E R S S R T L G C S W E F I P V D D G W G E R P L

The amino acid sequence of the BoNT/A prototoxin is encoded  
 by nucleotides 358 to 4245 of the neurotoxin cDNA sequence,  
 set forth herein below as SEQ ID NO: 10.

30 aagcttctaa atttaaatta ttaagtataa atccaaataa acaatatgtt caaaaacttg  
 atgaggtaat aatttctgta ttagataata tggaaaaata tatagatata tctgaagata  
 atagattgca actaatagat aacaaaaata acgcaaagaa gatgataatt agtaatgata  
 tattttatttc caattgttta accctatctt ataacggtaa atatatatgt ttatctatga  
 35 aagatgaaaa ccataattgg atgatatgta ataatgatat gtcaaagtat ttgtatttat  
 ggtcatttaa ataattaata atttaattaa ttttaaatat tataagaggt gttaaatatg  
 ccatttgta ataaacaatt taattataaa gatcctgtaa atgggtgttg tattgcttat  
 ataaaaattc caaatgcagg acaaatgcaa ccagtaaaag cttttaaaat tcataataaa  
 atatgggtta ttccagaaag agatacattt acaaatcctg aagaaggaga tttaaatcca  
 40 ccaccagaag caaaacaagt tccagtttca tattatgatt caacatattt aagtacagat  
 aatgaaaaag ataattattt aaagggaggt acaaaattat ttgagagaat ttattcaact  
 gatcttgga gaatgttggt aacatcaata gtaaggggaa taccattttg ggggtggaagt  
 acaatagata cagaattaaa agttattgat actaattgta ttaatgtgat acaaccagat  
 ggtagttata gatcagaaga acttaatcta gtaataatag gaccctcagc tgatattata  
 45 cagtttgaat gtaaaagctt tggacatgaa gttttgaatc ttacgcgaaa tggttatggc  
 tctactcaat acattagatt tagcccagat tttacatttg gttttgagga gtcacttgaa

5 gttgatacaa atcctctctttt aggtgcaggc aaatttgcta cagatccagc agtaacatta  
 gcacatgaac ttatacatgc tggacataga ttatatggaa tagcaattaa tccaaatagg  
 gtttttaaaag taaatactaa tgcctattat gaaatgagtg ggtagaagt aagctttgag  
 gaacttagaa catttggggg acatgatgca aagtttatag atagtttaca ggaaaacgaa  
 10 tttcgtctat attattataa taagtttaaa gatatagcaa gtacacttaa taaagctaaa  
 tcaatagtag gtactactgc ttcattacag tatatgaaaa atgtttttta agagaaatat  
 ctccatctcg aagatacatc tggaaaattt tcggtagata aattaaaatt tgataagtta  
 taaaaaatgt taacagagat ttacacagag gataattttg ttaagttttt taaagtactt  
 aacagaaaaa catatttgaa ttttgataaa gccgtattta agataaatat agtacctaag  
 gtaaattaca caatatatga tggatttaaat ttaagaaata caaatttagc agcaaacttt  
 15 aatggtcaaa atacagaaat taataatatg aattttacta aactaaaaaa ttttactgga  
 ttgtttgaat tttataagtt gctatgtgta agagggataa taacttctaa aactaaatca  
 ttagataaag gatacaataa ggcattaaat gatttatgta tcaaagttaa taattgggac  
 ttgtttttta gtccttcaga agataatttt actaatgatc taaataaagg agaagaaatt  
 acatctgata ctaatataga agcagcagaa gaaaatatta gtttagattt aatacaacaa  
 20 tattatttaa cctttaattt tgataatgaa cctgaaaata tttcaataga aaatctttca  
 agtgacatta taggccaatt agaacttatg cctaatatag aaagatttcc taatggaaaa  
 aagtatgagt tagataaata tactatgttc cattatcttc gtgctcaaga atttgaacat  
 ggtaaatcta ggattgcttt aacaaattct gttaacgaag cattattaaa tcctagtcgt  
 gtttatacat ttttttcttc agactatgta aagaaagtta ataaagctac ggaggcagct  
 25 atgttttttag gctgggtaga acaattagta tatgatttta ccgatgaaac tagcgaagta  
 agtactacgg ataaaattgc ggatataact ataattattc catatatagg acctgcttta  
 aatataggta atatgttata taaagatgat tttgtaggtg ctttaatat ttcaggagct  
 gttattctgt tagaatttat accagagatt gcaatacctg tattaggtac ttttgcactt  
 gtatcatata ttgcgaataa ggttctaacc gttcaaaca tagataatgc ttttaagtaa  
 30 agaaatgaaa aatgggatga ggtctataaa tatatagtaa caaattgggt agcaaaggtt  
 aatacacaga ttgatcta atagaaaaaa atgaaagaag ctttagaaaa tcaagcagaa  
 gcaacaaagg ctataataaa ctatcagtat aatcaatata ctgaggaaga gaaaaataat  
 attaatttta atattgatga ttttaagttcg aaacttaatg agtctataaa taaagctatg  
 attaataata ataaattttt gaatcaatgc tctgtttcat atttaatgaa ttctatgatc  
 35 ccttatgggtg ttaaacgggt agaagatttt gatgctagtc ttaaagatgc attattaag  
 tatatatatg ataataagagg aactttaatt ggtcaagtag atagattaaa agataaagtt  
 aataatacac ttagtacaga tatacctttt cagctttcca aatacgtaga taatcaaaga  
 ttattatcta catttactga atatatatag aatattatta atacttctat attgaattta  
 agatatgaaa gtaatcattt aatagactta tctaggtatg catcaaaaat aaatattgggt  
 40 agtaaagtaa attttgatcc aatagataaa aatcaaattc aattatttaa tttagaaagt  
 agtaaaattg aggtaatttt aaaaaatgct attgtatata atagtatgta tgaaaatttt  
 agtactagct tttggataag aattcctaag tattttaaca gtataagttt aaataatgaa  
 tatacaataa taaattgtat ggaaaataat tcaggatgga aagtatcact taattatgggt  
 gaaataatct ggactttaca ggatactcag gaaataaaac aaagagtagt ttttaaatac  
 45 agtcaaatga ttaatatatc agattatata aacagatgga tttttgtaac tatcactaat  
 aatagattaa ataactctaa aatttatata aatggaagat taatagatca aaaaccaatt  
 tcaaatttag gtaatattca tgctagtaat aatataatgt ttaaattaga tggttgtaga  
 gatacacata gatataattt gataaaatat tttaatcttt ttgataagga attaaatgaa  
 aaagaaatca aagatttata tgataatcaa tcaaattcag gtatttttaa agacttttgg  
 50 ggtgattatt tacaatatga taaaccatac tatatgttaa atttatatga tccaaataaa  
 tatgtcgatg taaataatgt aggtattaga ggttatatgt atcttaaagg gcctagaggt  
 agcgtaatga ctacaaacat ttattttaaat tcaagtttgt atagggggac aaaatttatt  
 ataaaaaat atgcttctgg aaataaagat aatattgtta gaaataatga tcgtgtatat

5   attaatgtag tagttaaaaa taaagaatat aggttagcta ctaatgcatc acaggcaggc  
      gtagaaaaaa tactaagtgc attagaaata cctgatgtag gaaatctaag tcaagtagta  
      gtaatgaagt caaaaaatga tcaaggaata acaataaat gcaaaatgaa tttaacaagat  
      aataatggga atgatatagg ctttatagga tttcatcagt ttaataatat agctaaacta  
      gtagcaagta attggtataa tagacaaata gaaagatcta gtaggacttt gggttgctca  
 10   tgggaattta ttcctgtaga tgatggatgg ggagaaaggc cactgtaatt aatctcaaac  
      tacatgagtc tgtcaagaat tttctgtaaa catccataaa aattttaaaa ttaatatgtt  
      taagaataac tagatatgag tattgtttga actgcccctg tcaagtagac aggtaaaaaa  
      ataaaaatta agatactatg gtctgatttc gatattctat cggagtcaga ccttttaact  
      tttcttgat cctttttgta ttgtaaaact ctatgtattc atcaattgca agttccaatt  
 15   agtcaaaatt atgaaacttt ctaagataat acattttctga ttttataatt tcccaaaatc  
      cttccatagg accattatca atacatctac caactcgaga catactttga gttgcgccta  
      tctcattaag tttattcttg aaagatttac ttgtatattg aaaaccgcta tcaactgtgaa  
      aaagtggact agcatcagga ttggaggtaa ctgctttatc aaaggtttca aagacaagga  
      cgttgttatt tgattttcca agtacatagg aaataatgct attatcatgc aaatcaagta  
 20   tttactcaa gtacgccttt gtttcgctg ttaac

Of course, three distinct domains analogous to those  
 described above for BoNT/A exist for all the BoNT subtypes  
 as well as for TeNT neurotoxin; an alignment of the amino  
 25   acid sequences of these holotoxins will reveal the sequence  
 coordinates for these other neurotoxin species.

Preferably, the translocation element and the binding  
 element of the compositions of the present invention are  
 separated by a spacer moiety that facilitates the binding  
 30   element's binding to the desired cell surface receptor. Such  
 a spacer may comprise, for example, a portion of the BoNT Hc  
 sequence (so long as the portion does not retain the ability  
 to bind to motor neurons or sensory afferent neurons),  
 another sequence of amino acids, or a hydrocarbon moiety.

35   The spacer moiety may also comprise a proline, serine,  
 threonine and/or cysteine-rich amino acid sequence similar  
 or identical to a human immunoglobulin hinge region. In a  
 preferred embodiment, the spacer region comprises the amino  
 acid sequence of an immunoglobulin  $\gamma 1$  hinge region; such a  
 40   sequence has the sequence (from N terminus to C terminus):

5 EPKSCDKTHTCPPCP (SEQ ID NO:11)

It will be understood that none of the examples or embodiments described herein are to be construed as limiting the scope of the invention, which is defined solely by the claims that conclude this specification.

10

Example 1:

An agent for the treatment of acute pancreatitis is constructed as follows.

15 A culture of *Clostridium botulinum* is permitted to grown to confluence. The cells are then lysed and total RNA is extracted according to conventional methods and in the presence of an RNase inhibitor. The RNA preparation is then passed over a oligo(dT) cellulose column, the polyadenylated  
20 messenger RNA is permitted to bind, and the column is washed with 5-10 column volumes of 20 mM Tris pH 7.6, 0.5 M NaCl, 1 mM EDTA (ethylenediamine tetraacetic acid), 0.1% (w/v) SDS (sodium dodecyl sulfate). Polyadenylated RNA is then eluted with 2-3 column volumes of STE (10 mM Tris (pH 7.6), 1 mM  
25 EDTA, 0.05% (w/v) SDS). The pooled mRNA is then precipitated in 2 volumes of ice cold ethanol, pelleted in a centrifuge at 10,000 x g for 15 minutes, then redissolved in a small volume of STE.

The BoNT/A mRNA is used as a template for DNA synthesis  
30 using Moloney murine leukemia virus reverse transcriptase (MMLV-RT), then the L chain and then H<sub>N</sub> chain of the neurotoxin is amplified from the cDNA by the polymerase chain reaction (PCR) using appropriate oligonucleotide primers whose sequences are designed based on the BoNT/A



5 neurotoxin cDNA sequence of SEQ ID NO: 9. These procedures are performed using the standard techniques of molecular biology as detailed in, for example, Sambrook et al., already incorporated by reference herein. The primer defining the beginning of the coding region (5'side of the L chain fragment) is given a StuI site. The PCR primer  
10 defining the 3' end of the HN-encoding domain has the following features (from 3' to 5'): a 5' region sufficiently complementary to the 3' end of the HN-encoding domain to anneal thereto under amplification conditions, a nucleotide  
15 sequence encoding the human immunoglobulin hinge region  $\gamma_1$  (SEQ ID NO:11), a nucleotide sequence encoding the human CCK-8 octapeptide (SEQ ID NO:6), and a unique restriction endonuclease cleavage site.

The PCR product (termed BoNT/A<sup>L-HN $\gamma$ -CCK</sup>) is purified by  
20 agarose gel electrophoresis, and cloned into a pBluescript II SK vector. The resulting plasmid is used to transform competent *E. coli* cells, and a preparation of the resulting plasmid is made. The BoNT/A<sup>L-HN $\gamma$ -CCK</sup> fragment is excised from the pBluescript vector and cloned into a mammalian  
25 expression vector immediately downstream of a strong promoter. The resulting vector is used to transfect a culture of the appropriate host cell, which is then grown to confluence. Expression of the BoNT/A<sup>L-HN $\gamma$ -CCK</sup> polypeptide is induced, and the cells are lysed. The polypeptide is first  
30 purified by gel exclusion chromatography, the fractions containing the recombinant therapeutic agent are pooled, then the BoNT/A<sup>L-HN $\gamma$ -CCK</sup> polypeptide is further purified using

5 an anti-Ig affinity column wherein the antibody is directed to the  $\gamma_1$  hinge region of a human immunoglobulin.

Example 2: Method of Treating a Patient Suffering from

10 Acute Pancreatitis

A therapeutically effective amount of the BoNT/AL-HN- $\gamma$ -CCK agent constructed and purified as set forth in Example 1 is formulated in an acceptable infusion solution. Properties  
15 of pharmacologically acceptable infusion solutions, including proper electrolyte balance, are well known in the art. This solution is provided intravenously to a patient suffering from acute pancreatitis on a single day over a period of one to two hours. Additionally, the patient is  
20 fed intravenously on a diet low in complex carbohydrates, complex fats and proteins.

At the beginning of treatment, the patient's pancreas shows signs of autodigestion, as measured by blood amylase levels. After the treatment regimen, autodigestion has  
25 ceased, and the patient's pancreas has stabilized.

Example 3: Alternative Treatment Method

In this example, a patient suffering from acute  
30 pancreatitis is treated as in Example 2, with, the therapeutic agent given continuously over a period of two weeks. After the treatment regimen, autodigestion has ceased, and the patient's pancreas has stabilized.

5 Example 4: Alternative Treatment Method

In this example, a patient suffering from acute pancreatitis is given a single pharmacologically effective amount of the therapeutic agent of Example 1 by parenteral administration.

10 Two days after the treatment regimen, autodigestion has ceased and the patient's pancreas has stabilized.

15

It will be understood that the present invention is not to be limited by the embodiments and examples described herein, and that the invention is defined solely by the claims that conclude this specification.

5

## CLAIMS

What is claimed is:

1. A composition for the treatment of acute pancreatitis  
10 in a mammal comprising,
  - a. a first element comprising a binding element able to specifically bind a pancreatic cell surface marker under physiological conditions,
  - b. a second element comprising a translocation  
15 element able to facilitate the transfer of a polypeptide across a vesicular membrane, and
  - c. a third element comprising a therapeutic element able, when present in the cytoplasm of a pancreatic cell, to inhibit enzymatic secretion by  
20 said pancreatic cell.
2. The composition of claim 1 wherein said pancreatic cell is an acinar cell and said cell surface marker is a CCK receptor.
- 25 3. The composition of claim 1 wherein said therapeutic element will cleave a SNARE protein and cleavage of said SNARE protein inhibits said secretion.
- 30 4. The composition of claim 3 wherein said SNARE protein is selected from the group consisting of syntaxin, SNAP-25 and VAMP.

- 5 5. The composition of claim 2 wherein said therapeutic  
element will cleave a SNARE protein, wherein cleavage  
of said SNARE protein inhibits said secretion.
6. The composition of claim 5 wherein said SNARE protein  
10 is selected from the group consisting of syntaxin,  
SNAP-25 and VAMP.
7. The composition of claim 5 wherein said CCK receptor is  
the human CCK A receptor.
- 15 8. The composition of claim 5 wherein said binding element  
comprises an amino acid sequence consisting of SEQ ID  
NO: 6.
- 20 9. The composition of claim 8 wherein said binding element  
comprises an amino acid sequence consisting of SEQ ID  
NO: 5.
10. The composition of claim 9 wherein said binding element  
25 comprises an amino acid sequence consisting of SEQ ID  
NO: 4.
11. The composition of claim 10 wherein said binding  
element comprises an amino acid sequence consisting of  
30 SEQ ID NO: 3.
12. The composition of claim 11 wherein said binding  
element comprises an amino acid sequence consisting of  
SEQ ID NO: 2.

5

13. The composition of claim 1 wherein said composition further comprises a spacer moiety separating said binding element from said translocation element.

10

14. The composition of claim 13 wherein said spacer moiety comprises a moiety selected from the group consisting of a hydrocarbon, a polypeptide other than an immunoglobulin hinge region, and a proline-containing polypeptide identical or analogous to an immunoglobulin hinge region.

15

15. The composition of claim 14 wherein said spacer moiety comprises a proline-containing polypeptide identical or analogous to an immunoglobulin hinge region.

20

16. The composition of claim 15 wherein said polypeptide comprises an amino acid sequence consisting of SEQ ID NO:11.

25

17. The composition of claim 7 wherein said composition further comprises a spacer moiety separating said binding element from said translocation element.

30

18. The composition of claim 17 wherein said spacer moiety comprises a moiety selected from the group consisting of a hydrocarbon, a polypeptide other than an immunoglobulin hinge region, and a proline-containing polypeptide identical or analogous to an immunoglobulin hinge region.

5

19. The composition of claim 18 wherein said spacer moiety comprises a proline-containing polypeptide identical or analogous to an immunoglobulin hinge region.

10

20. The composition of claim 19 wherein said polypeptide comprises an amino acid sequence consisting of SEQ ID NO:11.

15

21. The composition of claim 8 wherein said composition further comprises a spacer moiety separating said binding element from said translocation element.

20

22. The composition of claim 17 wherein said spacer moiety comprises a moiety selected from the group consisting of a hydrocarbon, a polypeptide other than an immunoglobulin hinge region, and a proline-containing polypeptide identical or analogous to an immunoglobulin hinge region.

25

23. The composition of claim 18 wherein said spacer moiety comprises a proline-containing polypeptide identical or analogous to an immunoglobulin hinge region.

30

24. The composition of claim 19 wherein said polypeptide comprises an amino acid sequence consisting of SEQ ID NO:11.

25. A method for the treatment of a mammal suffering from acute pancreatitis comprising:

5 administering to said patient a pharmaceutically  
effective amount of a composition comprising a first  
element comprising a binding element able to  
specifically bind a pancreatic cell surface marker  
under physiological conditions, a second element  
10 comprising a translocation element able to facilitate  
the transfer of a polypeptide across a vesicular  
membrane, and a third element comprising a therapeutic  
element able, when present in the cytoplasm of a  
pancreatic cell, to inhibit enzymatic secretion by said  
15 pancreatic cell.

26. The method of claim 25 wherein said pancreatic cell is  
an acinar cell and said cell surface marker is a CCK  
receptor.

27. The method of claim 26 wherein said therapeutic element  
will cleave a SNARE protein and cleavage of said SNARE  
protein inhibits said secretion.

28. The method of claim 27 wherein said SNARE protein is  
selected from the group consisting of syntaxin, SNAP-  
25, and VAMP.

29. The method of claim 28 wherein said CCK receptor is the  
30 human CCK A receptor.

30. The method of claim 29 wherein said binding element  
comprises an amino acid sequence consisting of SEQ ID  
NO: 6.



5

31. The method of claim 25 wherein said composition further comprises a spacer moiety separating said binding element from said translocation element.

10

32. The method of claim 31 wherein said spacer moiety comprises a moiety selected from the group consisting of a hydrocarbon, a polypeptide other than an immunoglobulin hinge region, and a proline-containing polypeptide identical or analogous to an immunoglobulin hinge region.

15

33. The method of claim 28 wherein said composition further comprises a spacer moiety separating said binding element from said translocation element.

20

34. The method of claim 33 wherein said spacer moiety comprises a moiety selected from the group consisting of a hydrocarbon, a polypeptide other than an immunoglobulin hinge region, and a proline-containing polypeptide identical or analogous to an immunoglobulin hinge region.

25

35. The method of claim 30 wherein said composition further comprises a spacer moiety separating said binding element from said translocation element.

30

36. The method of claim 35 wherein said spacer moiety comprises a moiety selected from the group consisting of a hydrocarbon, a polypeptide other than an

5 immunoglobulin hinge region, and a proline-containing  
polypeptide identical or analogous to an immunoglobulin  
hinge region.

10 37. The method of claim 25 wherein said composition is  
formulated in an infusion solution, and is administered  
to said patient intravenously.

15 38. The method of claim 31 wherein said composition is  
formulated in an infusion solution, and is administered  
to said patient intravenously.

20 39. The method of claim 33 wherein said composition is  
formulated in an infusion solution, and is administered  
to said patient intravenously.

40. The method of claim 35 wherein said composition is  
formulated in an infusion solution, and is administered  
to said patient intravenously.

5

## ABSTRACT

Methods and compositions for the treatment of acute  
pancreatitis in a mammal. Particular compositions comprise  
a binding element, a translocation element, and a  
10 therapeutic element able to prevent accumulation of  
digestive enzymes within the pancreas.

As a below named inventor, I hereby declare that:

I believe I am the original and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled **METHODS AND COMPOSITIONS FOR THE TREATMENT OF PANCREATITIS** the specification of which

[illegible]

I acknowledge the duty to disclose information which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, §1.56(a). I hereby claim foreign priority benefits under 35 USC § 119(a)-(d) or §365(b) of any foreign application(s) for patent or inventor's certificate, or §365(a) of any PCT International application which designated at least one country other than the United States, listed below and have also identified below any foreign application for patent or inventor's certificate, or PCT International application having a filing date before that of the Prior Foreign Applications(s).

| Number | Country | Day/Month/Yr filed) | Priority Not Claimed |
|--------|---------|---------------------|----------------------|
|        |         |                     |                      |

I hereby claim the benefit under 35 USC §119 (e) of any United States provisional application(s) listed below.

| Application No. | Filing Date |
|-----------------|-------------|
| 1               | 1/1/2020    |
| 2               | 1/1/2020    |
| 3               | 1/1/2020    |
| 4               | 1/1/2020    |
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| 100             | 1/1/2020    |

I hereby claim the benefit under Title 35, United States Code, §120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application(s) in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, §1.56(a) which occurred between the filing date of the prior application and the national or PCT international filing date of this application:

| Application No. | Filing Date |
|-----------------|-------------|
| 1               | 1/1/2018    |
| 2               | 1/1/2018    |
| 3               | 1/1/2018    |
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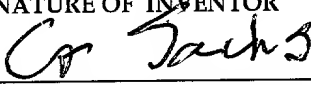
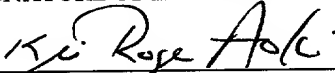
I hereby appoint **CARLOS A. FISHER, Registration No. 36,510** (to whom all communications are to be directed), and the below-named persons (of the same address) individually and collectively my attorneys to prosecute this application and to transact all

business in the Patent and Trademark Office connected therewith and with the resulting patent, with full power to appoint associate attorneys:

| <u>Name</u>    | <u>Registration No.</u> |
|----------------|-------------------------|
| Robert Baran   | 25,806                  |
| Martin A. Voet | 25,208                  |

of the following correspondence address: **Allergan, Inc., 2525 Dupont Drive, Irvine, CA. 92612**

I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under §1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

|   |   |                               |                                      |
|---|---|-------------------------------|--------------------------------------|
| <b>FULL NAME OF INVENTOR:</b>   |   |                               |                                      |
| <b>First Name:</b><br>George  | <b>Initial</b>                                | <b>Last Name</b><br>Sachs     |                                      |
| <b>RESIDENCE &amp; CITIZENSHIP</b>  |   |                               |                                      |
| <b>City</b><br>Encino   | <b>State or Foreign Country</b><br>California |                               | <b>Country of Citizenship</b><br>USA |
| <b>POST OFFICE ADDRESS</b>  |   |                               |                                      |
| <b>Post Office Address</b><br>17986 Boris Drive   | <b>City</b><br>Encino                         | <b>State or Country</b><br>CA | <b>Zip Code</b><br>91316             |
| <b>SIGNATURE OF INVENTOR</b><br> |   | <b>DATE:</b><br>4/8/99        |                                      |
| <b>FULL NAME OF INVENTOR:</b>   |   |                               |                                      |
| <b>First Name:</b><br>Kei   | <b>Initial</b><br>Roger                       | <b>Last Name</b><br>Aoki      |                                      |
| <b>RESIDENCE &amp; CITIZENSHIP</b>  |   |                               |                                      |
| <b>City</b><br>Coto de Caza   | <b>State or Foreign Country</b><br>California |                               | <b>Country of Citizenship</b><br>USA |
| <b>POST OFFICE ADDRESS</b>  |   |                               |                                      |
| <b>Post Office Address</b><br>2 Ginger Lily Court   | <b>City</b><br>Coto de Caza                   | <b>State or Country</b><br>CA | <b>Zip Code</b><br>92679             |
| <b>SIGNATURE OF INVENTOR</b><br> |   | <b>DATE:</b><br>4/7/99        |                                      |

662640-9261260

### 37 CFR § 1.56 Duty to Disclose Information Material to Patentability.

A patent by its very nature is affected with a public interest. The public interest is best served, and the most effective patent examination occurs when, at the time an application is being examined, the Office is aware of and evaluates the teachings of all information material to patentability. Each individual associated with the filing and prosecution of a patent application has a duty of candor and good faith in dealing with the Office, which includes a duty to disclose to the Office all information known to that individual to be material to patentability as defined in this section. The duty to disclose information exists with respect to each pending claim until the claim is cancelled or withdrawn from consideration, or the application becomes abandoned. Information material to the patentability of a claim that is cancelled or withdrawn from consideration need not be submitted if the information is not material to the patentability of any claim remaining under consideration in the application. There is no duty to submit information which is not material to the patentability of any existing claim. The duty to disclose all information known to be material to patentability is deemed to be satisfied if all information known to be material to patentability of any claim issued in a patent was cited by the Office or submitted to the Office in the manner prescribed by Section 1.97(b)-(d) and 1.98. However, no patent will be granted on an application in connection with which fraud on the Office was practiced or attempted or the duty of disclosure was violated through bad faith or intentional misconduct. The Office encourages applicants to carefully examine:

Prior art cited in search reports of a foreign patent office in a counterpart application, and

The closest information over which individuals associated with the filing or prosecution of a patent application believe any pending claim patentably defines, to make sure that any material information contained therein is disclosed to the Office.

Under this section, information is material to patentability when it is not cumulative to information already of record or being made of record in the application, and

It establishes, by itself or in combination with other information, a prima facie case of unpatentability of a claim; or

It refutes, or is inconsistent with, a position the applicant takes in:

Opposing an argument of unpatentability relied on by the Office, or

Asserting an argument of patentability.

A prima facie case of unpatentability is established when the information compels a conclusion that a claim is unpatentable under the preponderance of evidence, burden-of-proof standard, giving each term in the claim its broadest reasonable construction consistent with the specification, and before any consideration is given to evidence which may be submitted in an attempt to establish a contrary conclusion of patentability.

Individuals associated with the filing or prosecution of a patent application within the meaning of this section are:

Each inventor named in the application;

Each attorney or agent who prepares or prosecutes the application; and

Every other person who is substantively involved in the preparation or prosecution of the application and who is associated with the inventor, with the assignee or with anyone to whom there is an obligation to assign the application.

Individuals other than the attorney, agent or inventor may comply with this section by disclosing information to the attorney, agent, or inventor.

### 35 USC § 102. Conditions for Patentability; Novelty and Loss of Right to Patent

A person shall be entitled to a patent unless -

- (a) the invention was known or used by others in this country, or patented or described in a printed publication in this or a foreign country, before the invention thereof by the applicant for patent, or
- (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of the application for patent in the United States, or
- (c) he has abandoned the invention, or
- (d) the invention was first patented or caused to be patented, or was the subject of an inventor's certificate, by the applicant or his legal representatives or assigns in a foreign country prior to the date of the application for patent in this country on an application for patent or inventor's certificate filed more than twelve months before the filing of the application in the United States, or
- (e) the invention was described in a patent granted on an application for patent by another filed in the United States before the invention thereof by the applicant for patent, or on an international application by another who has fulfilled the requirements of paragraphs (1), (2), and (4) of section 371(c) of this title before the invention thereof by the applicant for patent, or
- (f) he did not himself invent the subject matter sought to be patented, or
- (g) before the applicant's invention thereof the invention was made in this country by another who had not abandoned, suppressed, or concealed it. In determining priority of invention there shall be considered not only the respective dates of conception and reduction to practice of the invention, but also the reasonable diligence of one who was first to conceive and last to reduce to practice, from a time prior to conception by the other.

### 35 USC § 103. Conditions for Patentability; Non-obvious Subject Matter

(a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negated by the manner in which the invention was made.

(b)

(1) Notwithstanding subsection (a), and upon timely election by the applicant for patent to proceed under this subsection, a biotechnological process using or resulting in a composition of matter that is novel under section 102 and nonobvious under subsection (a) of this section shall be considered nonobvious if -

- (A) claims to the process and the composition of matter are contained in either the same application for patent or in separate applications having the same effective filing date; and
- (B) the composition of matter, and the process at the time it was invented, were owned by the same person or subject to an obligation of assignment to the same person.

(2) A patent issued on a process under paragraph (1) -

- (A) shall also contain the claims to the composition of matter used in or made by that process,

or

- (B) shall, if such composition of matter is claimed in another patent, be set to expire on the same date as such other patent, notwithstanding section 154.

(3) For purposes of paragraph (1), the term "biotechnological process" means -

- (A) a process of genetically altering or otherwise inducing a single- or multi-celled organism to -
  - (i) express an exogenous nucleotide sequence,
  - (ii) inhibit, eliminate, augment, or alter expression of an endogenous nucleotide sequence, or

(C) a method of using a product produced by a process defined by subparagraph (A) or (B), or a combination of subparagraphs (A) and (B).



## SEQUENCE LISTING

<110> Kei Roger Aoki  
George Sachs

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|     | 50  |     |     |     |     | 55  |     |     |     | 60  |     |     |     |     |     |
| Tyr | Ile | Gln | Gln | Ala | Arg | Lys | Ala | Pro | Ser | Gly | Arg | Met | Ser | Ile | Val |
| 65  |     |     |     |     | 70  |     |     |     | 75  |     |     |     |     | 80  |     |
| Lys | Asn | Leu | Gln | Asn | Leu | Asp | Pro | Ser | His | Arg | Ile | Ser | Asp | Arg | Asp |
|     |     |     | 85  |     |     |     |     |     | 90  |     |     |     | 95  |     |     |
| Tyr | Met | Gly | Trp | Met | Asp | Phe | Gly | Arg | Arg | Ser | Ala | Glu | Glu | Tyr | Glu |
|     |     |     | 100 |     |     |     |     | 105 |     |     |     |     | 110 |     |     |
| Tyr | Pro | Ser |     |     |     |     |     |     |     |     |     |     |     |     |     |
|     |     |     | 115 |     |     |     |     |     |     |     |     |     |     |     |     |

<210> 2

<211> 58

<212> PRT

<213> Homo sapiens

<400> 2

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Val | Ser | Gln | Arg | Thr | Asp | Gly | Glu | Ser | Arg | Ala | His | Leu | Gly | Ala | Leu |
| 1   |     |     |     | 5   |     |     |     |     | 10  |     |     |     |     | 15  |     |
| Leu | Ala | Arg | Tyr | Ile | Gln | Gln | Ala | Arg | Lys | Ala | Pro | Ser | Gly | Arg | Met |
|     |     |     | 20  |     |     |     |     | 25  |     |     |     |     | 30  |     |     |
| Ser | Ile | Val | Lys | Asn | Leu | Gln | Asn | Leu | Asp | Pro | Ser | His | Arg | Ile | Ser |
|     |     | 35  |     |     |     | 40  |     |     |     |     |     | 45  |     |     |     |
| Asp | Arg | Asp | Tyr | Met | Gly | Trp | Met | Asp | Phe |     |     |     |     |     |     |
| 50  |     |     |     |     |     | 55  |     |     |     |     |     |     |     |     |     |

<210> 3

<211> 39

<212> PRT

<213> Homo sapiens

<400> 3  
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 1 5 10 15  
 Lys Asn Leu Gln Asn Leu Asp Pro Ser His Arg Ile Ser Asp Arg Asp  
 20 25 30  
 Tyr Met Gly Trp Met Asp Phe  
 35

<210> 4  
 <211> 33  
 <212> PRT  
 <213> Homo sapiens

<400> 4  
 Lys Ala Pro Ser Gly Arg Met Ser Ile Val Lys Asn Leu Gln Asn Leu  
 1 5 10 15  
 Asp Pro Ser His Arg Ile Ser Asp Arg Asp Tyr Met Gly Trp Met Asp  
 20 25 30  
 Phe

<210> 5  
 <211> 12  
 <212> PRT  
 <213> Homo sapiens

<400> 5  
 Ile Ser Asp Arg Asp Tyr Met Gly Trp Met Asp Phe  
 1 5 10

<210> 6  
 <211> 9  
 <212> PRT  
 <213> Homo sapiens

<400> 6  
 Arg Asp Tyr Met Gly Trp Met Asp Phe  
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<210> 7  
 <211> 448  
 <212> PRT  
 <213> Clostridium botulinum

<400> 7  
 Met Pro Phe Val Asn Lys Gln Phe Asn Tyr Lys Asp Pro Val Asn Gly  
 1 5 10 15  
 Val Asp Ile Ala Tyr Ile Lys Ile Pro Asn Ala Gly Gln Met Gln Pro  
 20 25 30  
 Val Lys Ala Phe Lys Ile His Asn Lys Ile Trp Val Ile Pro Glu Arg  
 35 40 45  
 Asp Thr Phe Thr Asn Pro Glu Gly Asp Leu Asn Pro Pro Pro Glu  
 50 55 60  
 Ala Lys Gln Val Pro Val Ser Tyr Tyr Asp Ser Thr Tyr Leu Ser Thr  
 65 70 75 80  
 Asp Asn Glu Lys Asp Asn Tyr Leu Lys Gly Val Thr Lys Leu Phe Glu  
 85 90 95

Arg Ile Tyr Ser Thr Asp Leu Gly Arg Met Leu Leu Thr Ser Ile Val  
 100 105 110  
 Arg Gly Ile Pro Phe Trp Gly Gly Ser Thr Ile Asp Thr Glu Leu Lys  
 115 120 125  
 Val Ile Asp Thr Asn Cys Ile Asn Val Ile Gln Pro Asp Gly Ser Tyr  
 130 135 140  
 Arg Ser Glu Glu Leu Asn Leu Val Ile Ile Gly Pro Ser Ala Asp Ile  
 145 150 155 160  
 Ile Gln Phe Glu Cys Lys Ser Phe Gly His Glu Val Leu Asn Leu Thr  
 165 170 175  
 Arg Asn Gly Tyr Gly Ser Thr Gln Tyr Ile Arg Phe Ser Pro Asp Phe  
 180 185 190  
 Thr Phe Gly Phe Glu Glu Ser Leu Glu Val Asp Thr Asn Pro Leu Leu  
 195 200 205  
 Gly Ala Gly Lys Phe Ala Thr Asp Pro Ala Val Thr Leu Ala His Glu  
 210 215 220  
 Leu Ile His Ala Gly His Arg Leu Tyr Gly Ile Ala Ile Asn Pro Asn  
 225 230 235 240  
 Arg Val Phe Lys Val Asn Thr Asn Ala Tyr Tyr Glu Met Ser Gly Leu  
 245 250 255  
 Glu Val Ser Phe Glu Glu Leu Arg Thr Phe Gly Gly His Asp Ala Lys  
 260 265 270  
 Phe Ile Asp Ser Leu Gln Glu Asn Glu Phe Arg Leu Tyr Tyr Tyr Asn  
 275 280 285  
 Lys Phe Lys Asp Ile Ala Ser Thr Leu Asn Lys Ala Lys Ser Ile Val  
 290 295 300  
 Gly Thr Thr Ala Ser Leu Gln Tyr Met Lys Asn Val Phe Lys Glu Lys  
 305 310 315 320  
 Tyr Leu Leu Ser Glu Asp Thr Ser Gly Lys Phe Ser Val Asp Lys Leu  
 325 330 335  
 Lys Phe Asp Lys Leu Tyr Lys Met Leu Thr Glu Ile Tyr Thr Glu Asp  
 340 345 350  
 Asn Phe Val Lys Phe Phe Lys Val Leu Asn Arg Lys Thr Tyr Leu Asn  
 355 360 365  
 Phe Asp Lys Ala Val Phe Lys Ile Asn Ile Val Pro Lys Val Asn Tyr  
 370 375 380  
 Thr Ile Tyr Asp Gly Phe Asn Leu Arg Asn Thr Asn Leu Ala Ala Asn  
 385 390 395 400  
 Phe Asn Gly Gln Asn Thr Glu Ile Asn Asn Met Asn Phe Thr Lys Leu  
 405 410 415  
 Lys Asn Phe Thr Gly Leu Phe Glu Phe Tyr Lys Leu Leu Cys Val Arg  
 420 425 430  
 Gly Ile Ile Thr Ser Lys Thr Lys Ser Leu Asp Lys Gly Tyr Asn Lys  
 435 440 445

&lt;210&gt; 8

&lt;211&gt; 423

&lt;212&gt; PRT

&lt;213&gt; Clostridium botulinum

&lt;400&gt; 8

Ala Leu Asn Asp Leu Cys Ile Lys Val Asn Asn Trp Asp Leu Phe Phe  
 1 5 10 15  
 Ser Pro Ser Glu Asp Asn Phe Thr Asn Asp Leu Asn Lys Gly Glu Glu  
 20 25 30  
 Ile Thr Ser Asp Thr Asn Ile Glu Ala Ala Glu Glu Asn Ile Ser Leu  
 35 40 45

Asp Leu Ile Gln Gln Tyr Tyr Leu Thr Phe Asn Phe Asp Asn Glu Pro  
 50 55 60  
 Glu Asn Ile Ser Ile Glu Asn Leu Ser Ser Asp Ile Ile Gly Gln Leu  
 65 70 75 80  
 Glu Leu Met Pro Asn Ile Glu Arg Phe Pro Asn Gly Lys Lys Tyr Glu  
 85 90 95  
 Leu Asp Lys Tyr Thr Met Phe His Tyr Leu Arg Ala Gln Glu Phe Glu  
 100 105 110  
 His Gly Lys Ser Arg Ile Ala Leu Thr Asn Ser Val Asn Glu Ala Leu  
 115 120 125  
 Leu Asn Pro Ser Arg Val Tyr Thr Phe Phe Ser Ser Asp Tyr Val Lys  
 130 135 140  
 Lys Val Asn Lys Ala Thr Glu Ala Ala Met Phe Leu Gly Trp Val Glu  
 145 150 155 160  
  
 Gln Leu Val Tyr Asp Phe Thr Asp Glu Thr Ser Glu Val Ser Thr Thr  
 165 170 175  
 Asp Lys Ile Ala Asp Ile Thr Ile Ile Ile Pro Tyr Ile Gly Pro Ala  
 180 185 190  
 Leu Asn Ile Gly Asn Met Leu Tyr Lys Asp Asp Phe Val Gly Ala Leu  
 195 200 205  
 Ile Phe Ser Gly Ala Val Ile Leu Leu Glu Phe Ile Pro Glu Ile Ala  
 210 215 220  
 Ile Pro Val Leu Gly Thr Phe Ala Leu Val Ser Tyr Ile Ala Asn Lys  
 225 230 235 240  
 Val Leu Thr Val Gln Thr Ile Asp Asn Ala Leu Ser Lys Arg Asn Glu  
 245 250 255  
 Lys Trp Asp Glu Val Tyr Lys Tyr Ile Val Thr Asn Trp Leu Ala Lys  
 260 265 270  
 Val Asn Thr Gln Ile Asp Leu Ile Arg Lys Lys Met Lys Glu Ala Leu  
 275 280 285  
 Glu Asn Gln Ala Glu Ala Thr Lys Ala Ile Ile Asn Tyr Gln Tyr Asn  
 290 295 300  
 Gln Tyr Thr Glu Glu Glu Lys Asn Asn Ile Asn Phe Asn Ile Asp Asp  
 305 310 315 320  
 Leu Ser Ser Lys Leu Asn Glu Ser Ile Asn Lys Ala Met Ile Asn Ile  
 325 330 335  
 Asn Lys Phe Leu Asn Gln Cys Ser Val Ser Tyr Leu Met Asn Ser Met  
 340 345 350  
 Ile Pro Tyr Gly Val Lys Arg Leu Glu Asp Phe Asp Ala Ser Leu Lys  
 355 360 365  
 Asp Ala Leu Leu Lys Tyr Ile Tyr Asp Asn Arg Gly Thr Leu Ile Gly  
 370 375 380  
 Gln Val Asp Arg Leu Lys Asp Lys Val Asn Asn Thr Leu Ser Thr Asp  
 385 390 395 400  
 Ile Pro Phe Gln Leu Ser Lys Tyr Val Asp Asn Gln Arg Leu Leu Ser  
 405 410 415  
 Thr Phe Thr Glu Tyr Ile Lys  
 420

&lt;210&gt; 9

&lt;211&gt; 382

&lt;212&gt; PRT

&lt;213&gt; Clostridium botulinum

&lt;400&gt; 9

Gln Leu Phe Asn Leu Glu Ser Ser Lys Ile Glu Val Ile Leu Lys Asn

|   |     |     |     |
|---|-----|-----|-----|
| 1   | 5   | 10  | 15  |
| Ala Ile Val Tyr Asn Ser Met Tyr Glu Asn Phe Ser Thr Ser Phe Trp |     |     |     |
|   | 20  | 25  | 30  |
| Ile Arg Ile Pro Lys Tyr Phe Asn Ser Ile Ser Leu Asn Asn Glu Tyr |     |     |     |
|   | 35  | 40  | 45  |
| Thr Ile Ile Asn Cys Met Glu Asn Asn Ser Gly Trp Lys Val Ser Leu |     |     |     |
|   | 50  | 55  | 60  |
| Asn Tyr Gly Glu Ile Ile Trp Thr Leu Gln Asp Thr Gln Glu Ile Lys |     |     |     |
| 65  | 70  | 75  | 80  |
| Gln Arg Val Val Phe Lys Tyr Ser Gln Met Ile Asn Ile Ser Asp Tyr |     |     |     |
|   | 85  | 90  | 95  |
| Ile Asn Arg Trp Ile Phe Val Thr Ile Thr Asn Asn Arg Leu Asn Asn |     |     |     |
|   | 100 | 105 | 110 |
| Ser Lys Ile Tyr Ile Asn Gly Arg Leu Ile Asp Gln Lys Pro Ile Ser |     |     |     |
|   | 115 | 120 | 125 |
| Asn Leu Gly Asn Ile His Ala Ser Asn Asn Ile Met Phe Lys Leu Asp |     |     |     |
| 130   | 135 | 140 |     |
| Gly Cys Arg Asp Thr His Arg Tyr Ile Trp Ile Lys Tyr Phe Asn Leu |     |     |     |
| 145   | 150 | 155 | 160 |
| Phe Asp Lys Glu Leu Asn Glu Lys Glu Ile Lys Asp Leu Tyr Asp Asn |     |     |     |
|   | 165 | 170 | 175 |
| Gln Ser Asn Ser Gly Ile Leu Lys Asp Phe Trp Gly Asp Tyr Leu Gln |     |     |     |
|   | 180 | 185 | 190 |
| Tyr Asp Lys Pro Tyr Tyr Met Leu Asn Leu Tyr Asp Pro Asn Lys Tyr |     |     |     |
|   | 195 | 200 | 205 |
| Val Asp Val Asn Asn Val Gly Ile Arg Gly Tyr Met Tyr Leu Lys Gly |     |     |     |
|   | 210 | 215 | 220 |
| Pro Arg Gly Ser Val Met Thr Thr Asn Ile Tyr Leu Asn Ser Ser Leu |     |     |     |
| 225   | 230 | 235 | 240 |
| Tyr Arg Gly Thr Lys Phe Ile Ile Lys Lys Tyr Ala Ser Gly Asn Lys |     |     |     |
|   | 245 | 250 | 255 |
| Asp Asn Ile Val Arg Asn Asn Asp Arg Val Tyr Ile Asn Val Val Val |     |     |     |
|   | 260 | 265 | 270 |
| Lys Asn Lys Glu Tyr Arg Leu Ala Thr Asn Ala Ser Gln Ala Gly Val |     |     |     |
|   | 275 | 280 | 285 |
| Glu Lys Ile Leu Ser Ala Leu Glu Ile Pro Asp Val Gly Asn Leu Ser |     |     |     |
|   | 290 | 295 | 300 |
| Gln Val Val Val Met Lys Ser Lys Asn Asp Gln Gly Ile Thr Asn Lys |     |     |     |
| 305   | 310 | 315 | 320 |
| Cys Lys Met Asn Leu Gln Asp Asn Asn Gly Asn Asp Ile Gly Phe Ile |     |     |     |
|   | 325 | 330 | 335 |
| Gly Phe His Gln Phe Asn Asn Ile Ala Lys Leu Val Ala Ser Asn Trp |     |     |     |
|   | 340 | 345 | 350 |
| Tyr Asn Arg Gln Ile Glu Arg Ser Ser Arg Thr Leu Gly Cys Ser Trp |     |     |     |
|   | 355 | 360 | 365 |
| Glu Phe Ile Pro Val Asp Asp Gly Trp Gly Glu Arg Pro Leu         |     |     |     |
| 370   | 375 | 380 |     |

&lt;210&gt; 10

&lt;211&gt; 4835

&lt;212&gt; DNA

&lt;213&gt; Clostridium botulinum

&lt;400&gt; 10

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atgaggtaat aatttctgta ttagataata tggaaaaata tatagatata tctgaagata

60  
120

|             |             |             |             |             |             |      |
|-------------|-------------|-------------|-------------|-------------|-------------|------|
| atagattgca  | actaatagat  | aacaaaaata  | acgcaaagaa  | gatgataatt  | agtaatgata  | 180  |
| tattttatttc | caattgttta  | accctatctt  | ataacggtaa  | atatatatgt  | ttatctatga  | 240  |
| aagatgaaaa  | ccataattgg  | atgatatgta  | ataatgatat  | gtcaaagtat  | ttgtattttat | 300  |
| ggtcattttaa | ataattaata  | atttaattaa  | ttttaaatat  | tataagaggt  | gttaaatatg  | 360  |
| ccatttggtta | ataaacaatt  | taattataaa  | gatcctgtaa  | atgggtgttg  | tattgcttat  | 420  |
| ataaaaaattc | caaatgcagg  | acaaatgcaa  | ccagtaaaaag | cttttaaaat  | tcataataaa  | 480  |
| atatgggtta  | ttccagaaaag | agatacattt  | acaaatcctg  | aagaaggaga  | tttaaattcca | 540  |
| ccaccagaag  | caaaacaagt  | tccagtttca  | tattatgatt  | caacatatatt | aagtacagat  | 600  |
| aatgaaaaaag | ataattattt  | aaagggagtt  | acaaaattat  | ttgagagaat  | ttattcaact  | 660  |
| gatcttgga   | gaatgttggt  | aacatcaata  | gtaaggggaa  | taccattttg  | gggtggaagt  | 720  |
| acaattagata | cagaatttaa  | agttattgat  | actaattgta  | ttaatgtgat  | acaaccagat  | 780  |
| ggtagttata  | gacagaaga   | acttaatcta  | gtaataatag  | gaccctcagc  | tgatattata  | 840  |
| cagtttgaat  | gtaaaagctt  | tggacatgaa  | gttttgaatc  | ttacgcgaaa  | tggttatggc  | 900  |
| tctactcaat  | acattagatt  | tagcccagat  | tttacatttg  | gttttgagga  | gtcacttgaa  | 960  |
| gttgatacaa  | atcctctttt  | aggtgcaggc  | aaatttgcta  | cagatccagc  | agtaacatta  | 1020 |
| gcacatgaac  | ttatacatgc  | tggacataga  | ttatatggaa  | tagcaattaa  | tccaaatagg  | 1080 |
| gttttttaaag | taaatactaa  | tgccctattat | gaaatgagtg  | ggtagaagt   | aagctttgag  | 1140 |
| gaacttagaa  | catttggggg  | acatgatgca  | aagtttatag  | atagttttaca | ggaaaacgaa  | 1200 |
| tttcgtctat  | attattataa  | taagtttaaa  | gatatagcaa  | gtacacttaa  | taaagctaaa  | 1260 |
| tcaatagtag  | gtactactgc  | ttcattacag  | tatatgaaaa  | atgtttttta  | agagaaatat  | 1320 |
| ctcctatctg  | aagatacatc  | tggaaaattt  | tcggtagata  | aattaaaatt  | tgataagtta  | 1380 |
| tacaaaatgt  | taacagagat  | ttacacagag  | gataattttg  | ttaagttttt  | taaagtactt  | 1440 |
| aacagaaaaa  | catatttgaa  | ttttgataaa  | gccgtattta  | agataaatat  | agtacctaag  | 1500 |
| gtaaattaca  | caatatatga  | tggatttaat  | ttaagaaata  | caaatttagc  | agcaaacctt  | 1560 |
| aatggtcaaa  | atacagaaa   | taataatatg  | aattttacta  | aactaaaaaa  | ttttactgga  | 1620 |
| ttgtttgaat  | tttataagtt  | gctatgtgta  | agagggataa  | taacttctaa  | aactaaatca  | 1680 |
| ttagataaag  | gatacaataa  | ggcatttaaat | gattttatgta | tcaaagttta  | taattgggac  | 1740 |
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| tattattttta | ccttttaattt | tgataatgaa  | cctgaaaata  | tttcaataga  | aaatctttca  | 1920 |
| agtgacatta  | taggccaatt  | agaacttatg  | cctaatatag  | aaagatttcc  | taatggaaaa  | 1980 |
| aagtatgagt  | tagataaata  | tactatgttc  | cattatcttc  | gtgctcaaga  | atttgaacat  | 2040 |
| ggtaaatcta  | ggattgcttt  | aacaaattct  | gttaacgaag  | cattatttaa  | tcctagtcgt  | 2100 |
| gtttatacat  | ttttttcttc  | agactatgta  | aagaaagtta  | ataaagctac  | ggaggcagct  | 2160 |
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| agaaatgaaa  | aatgggatga  | ggtctataaa  | tatatagtaa  | caaattgggtt | agcaaaggtt  | 2520 |
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| gcaacaaagg  | ctataataaa  | ctatcagtat  | aatcaatata  | ctgaggaaga  | gaaaaataat  | 2640 |
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| attaatataa  | ataaattttt  | gaatcaatgc  | tctgtttcat  | atttaatgaa  | ttctatgatc  | 2760 |
| ccttatgggtg | ttaaaacggtt | agaagatttt  | gatgctagtc  | ttaaagatgc  | attattaaag  | 2820 |
| tatatatatg  | ataatagagg  | aacttttaatt | gggtcaagtag | atagattaaa  | agataaagtt  | 2880 |
| aataatacac  | ttagtacaga  | tatacctttt  | cagctttcca  | aatacgtaga  | taatcaaaga  | 2940 |
| ttattatcta  | catttactga  | atatattaag  | aatattatta  | atacttctat  | attgaattta  | 3000 |
| agatatgaaa  | gtaatcattt  | aatagactta  | tctagggtatg | catcaaaaat  | aatatttggt  | 3060 |
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| agtactagct  | tttggataag  | aattcctaag  | tatttttaaca | gtataagtct  | aaataatgaa  | 3240 |
| tatacaataa  | taaattgtat  | ggaaaataat  | tcaggatgga  | aagtatcact  | taattatggt  | 3300 |
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| agtcaaatga  | ttaatatatc  | agattatata  | aacagatgga  | tttttgtaac  | tatcactaat  | 3420 |
| aatagattaa  | ataactctaa  | aatttatata  | aatggaagat  | taatagatca  | aaaaccaatt  | 3480 |
| tcaaatttag  | gtaatattca  | tgctagtaaat | aatataatgt  | ttaaattaga  | tgggtgtaga  | 3540 |

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&lt;210&gt; 11

&lt;211&gt; 15

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 11

Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro  
1 5 10 15